

of the GABA_A receptor cluster (Meguro et al. 1997); another group found these genes to be biallelically expressed (Gabriel et al. 1998). In both reports, the same imprinted and nonimprinted controls from human 15q11-13 were used.

The finding of biallelic expression in most fetal tissues and of paternal expression in the central nervous system, taken together with the absence of primary sequence mutations in a large panel of patients with SRS, argues against a major role for *GRB10* in SRS. However, with the identification of a maternally expressed isoform in skeletal muscle and the possibility that epigenetic alterations affecting *GRB10* activity still remain, *GRB10* may yet be involved in at least a subset of patients with SRS.

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References

- Angrist M, Bolk S, Bentley K, Nallasam S, Hallushka MK, Charkravarti A (1998) Genomic structure of the gene for the SH2 and pleckstrin homology domain-containing protein GRB10 and evaluation of its role in Hirschsprung disease. *Oncogene* 17:3065–3070
- Blagitko N, Mergenthaler S, Schulz U, Wollmann HA, Craigen W, Eggermann T, Ropers HH, Kalschauer VM (2000) Human GRB10 is imprinted and expressed from the paternal and maternal allele in a highly tissue- and isoform-specific fashion. *Hum Mol Genet* 9:1587–1595
- Gabriel JM, Higgins MJ, Gebuhr TC, Shows TB, Saitoh S, Nicholls RD (1998) A model system to study genomic

imprinting of human genes. *Proc Natl Acad Sci USA* 95: 14857–14862

- Hitchins MP, Monk D, Bell GM, Ali Z, Preece MA, Stanier P, Moore GE. Maternal repression of the human *GRB10* gene in the developing central nervous system: evaluation of the role for GRB10 in Silver-Russell syndrome. *Eur J Hum Genet* (in press)
- Joyce CA, Sharp A, Walker JA, Bullman H, Temple K (1999) Duplication of 7p12.1-p13 including *GRB10* and *IGFBP1* in a mother and daughter with features of Silver-Russell syndrome. *Hum Genet* 105:273–280
- Meguro M, Mitsuya K, Sui H, Shigenami K, Kugoh H, Nakao M, Oshimura M (1997) Evidence for uniparental, paternal expression of the human GABA_A receptor subunit genes, using microcell-mediated chromosome transfer. *Hum Mol Genet* 6:2127–2133
- Monk D, Wakeling EL, Proud V, Hitchins M, Abu-Amero SN, Stanier P, Preece MA, Moore GE (2000) Duplication of 7p11.2-p13, including GRB10, in Silver-Russell syndrome. *Am J Hum Genet* 66:36–46
- Yoshihashi H, Maeyama K, Kosaki K, Ogata T, Tsukahara M, Goto Y, Hata J, Matsuo N, Smith RJ, Kosaki K (2000) Imprinting of human GRB10 and its mutations in two patients with Russell-Silver syndrome. *Am J Hum Genet* 67: 476–482

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Reply to Mergenthaler et al.

To the Editor:

The thoughtful letter from Mergenthaler et al. (2001) illustrates the complex nature of the imprinting status of human GRB10. Blagitko et al. (2000) and Mergenthaler et al. (2001) have presented convincing data that human GRB10 is expressed from the paternal allele in fetal brain. This represents the first example of homologous genes being reciprocally imprinted in humans and mice. In their letter, Mergenthaler et al. make a number of valid points in relation to our report on imprinting of GRB10 as it relates to Russell-Silver syndrome (RSS). However, they also raise several issues that merit clarification.

We believe that the title of their letter, “Conflicting Reports of Imprinting Status of Human GRB10 in Developing Brain,” overstates the extent of controversy. We would like to point out that we specifically concluded, in our report, only that human GRB10 is monoallelically

expressed in the fetal brain, because we were unable to determine the parental origin of the expressed allele without having parental genomic DNA (Yoshihashi et al. 2000). As an alternative, we evaluated the somatic cell hybrid system and concluded that the maternal allele is expressed in that experimental system.

On the basis of paternal allele-specific expression of GRB10 in the fetal brain, Mergenthaler et al. (2001) concluded that GRB10 does not play a significant role in the pathogenesis of RSS, with rather little attention to their own finding that one isoform (gamma 1) is expressed from the maternal allele in skeletal muscle (Blagitko et al. 2000). Because northern blot analyses have indicated that GRB10 mRNA is expressed much more abundantly in muscle than in brain (Liu and Roth 1995), the importance of the maternal allele-specific expression in the skeletal muscle should not be overlooked. The tissue-specific reciprocal imprinting pattern of GRB10 in muscle and brain indeed could account for the characteristic disproportionate growth retardation of the head and the body sizes observed among patients with RSS. This peculiar growth pattern is referred to as relative macrocephaly and is characterized by greater growth of the head in comparison with severely retarded growth of the body. Tissue-specific reciprocal imprinting could exert differential effects on the growth of muscle, brain, and possibly other tissues in patients with RSS who have maternal UPD7 or duplication of 7p11.2-p13. Maternally expressed muscle transcripts would be overexpressed, whereas paternally expressed brain transcripts would not be expressed in excess. If GRB10 indeed functions as a growth suppressor (O'Neill et al. 1996), the resultant phenotype would be sparing of brain size—that is, relative macrocephaly.

We screened samples from 58 patients with RSS and identified two Japanese patients with a P95S change, which was not present among 100 normal Japanese controls (Yoshihashi et al. 2000). Subsequent screening of >300 normal Japanese individuals by Yamasaki et al. (2000) identified two individuals with the P95S sequence, suggesting the possibility that this may represent a rare polymorphism in the Japanese population. Unfortunately, the parental origin of the P95S allele in the two phenotypically normal individuals could not be determined, and thus it is not possible to make inferences on the likely expression or silence of this altered allele. It will be important to directly compare the functional properties of GRB10 containing either a P or an S residue at position 95, and these studies currently are in progress. Together with the lack of GRB10 mutations in 50 German patients (Blagitko et al. 2000) and in 31 English patients (Mergenthaler et al. 2001), we agree with the conclusion that mutations in the coding sequence of GRB10 are rare, if they occur at all, as determinants of RSS. Moreover, we hope that analysis of the complex imprinting mechanism

of GRB10 and its flanking region at 7p12 will provide further insight into the pathogenesis of RSS.

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References

- Blagitko N, Mergenthaler S, Schulz U, Wollmann HA, Craigen W, Eggermann T, Ropers HH, Kalscheuer VM (2000) Human GRB10 is imprinted and expressed from the paternal and maternal allele in a highly tissue- and isoform-specific fashion. *Hum Mol Genet* 9:1587–1595
- Liu F, Roth RA (1995) Grb-IR: a SH2-domain-containing protein that binds to the insulin receptor and inhibits its function. *Proc Natl Acad Sci USA* 92:10287–10291
- Mergenthaler S, Hitchens M P, Blagitko-Dorfs N, Blagitko-Dorfs N, Monk D, Wollmann HA, Ranke MB, Ropers H, Apostolidou S, Stainer P, Preece MA, Eggermann T, Kalscheuer VM, Moore G (2001) Conflicting reports of imprinting status of human GRB10 in developing brain. *Am J Hum Genet* 68: 543–544 (in this issue)
- O'Neill TJ, Rose DW, Pillay TS, Hotta K, Olefsky JM, Gustafson TA (1996) Interaction of a GRB-IR splice variant (a human GRB10 homolog) with the insulin and insulin-like growth factor I receptors: evidence for a role in mitogenic signaling. *J Biol Chem* 271:22506–22513
- Yamasaki K, Hayashida Y, Tachibana T, Kishino Y, Niikawa N (2000) Construction of an imprinted status transcript map flanking MEST/PEG1 at 7q32. *Am J Hum Genet* 67:1010A
- Yoshihashi H, Maeyama K, Kosaki R, Ogata T, Tsukahara M, Goto Y, Hata J, Matsuo N, Smith RJ, Kosaki K (2000) Imprinting of human GRB10 and its mutations in two patients with Russell-Silver syndrome. *Am J Hum Genet* 67:476–482

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